

Fig. 1

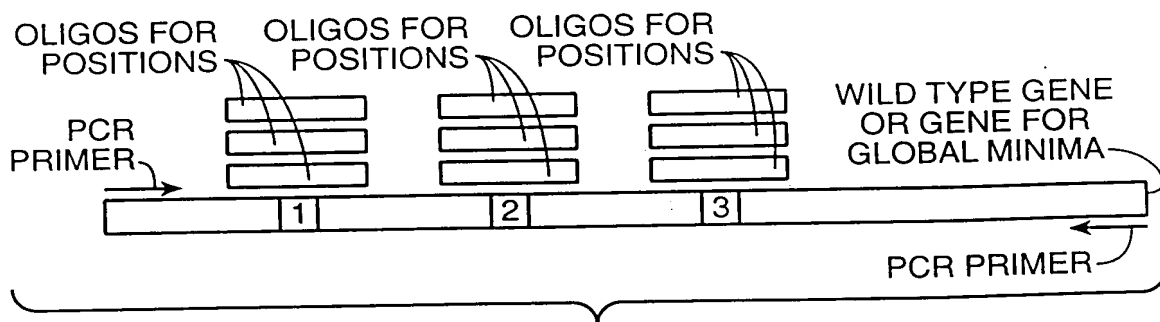
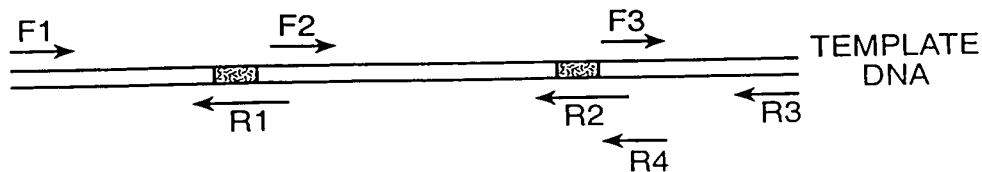


Fig. 2

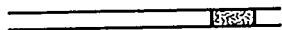
BLACK BOX =
REGION TO
BE MUTATED



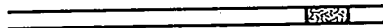
STEP 1: SET UP 3 PCR REACTIONS:

PRODUCTS:

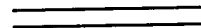
TUBE 1:



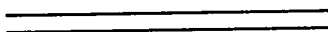
TUBE 2:



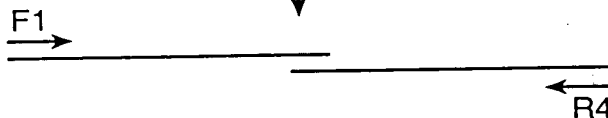
TUBE 3:



STEP 2: SET UP PCR REACTION WITH PRODUCTS OF TUBE 1 +
PRODUCTS TUBE 2 + F1 + R4.



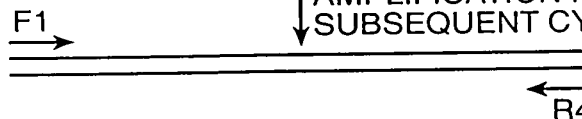
↓ HEAT + ANNEAL PHASE OF PCR,



↓ SYNTHESIS PHASE OF PCR,

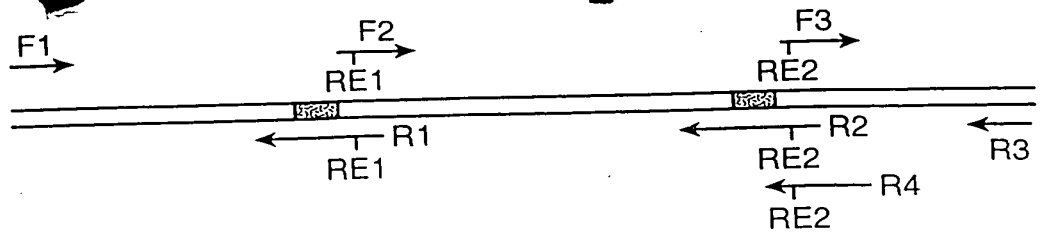


↓ AMPLIFICATION PHASE DURING
SUBSEQUENT CYCLES USING F1 + R4.



STEP 3: REPEAT STEP 2 USING PRODUCT FROM STEP 2 + PRODUCT
FROM STEP 1, TUBE 3 + PRIMERS F1 + R3.

Fig. 3



STEP 1: SET UP 3 PCR REACTIONS:

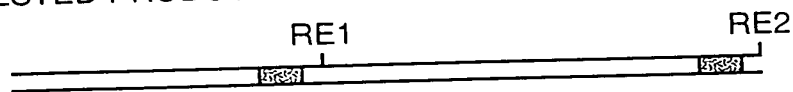
TUBE 1:

TUBE 2:

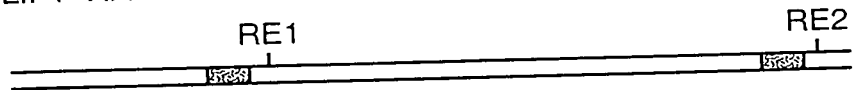
TUBE 3:

STEP 2: DIGEST PRODUCTS FROM STEP 1 WITH SUITABLE RESTRICTION ENDONUCLEASES.

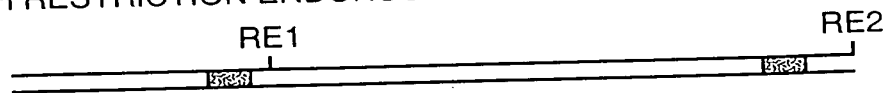
STEP 3: LIGATE DIGESTED PRODUCT FROM STEP 2, TUBE 2 WITH DIGESTED PRODUCT FROM STEP 2, TUBE 1.



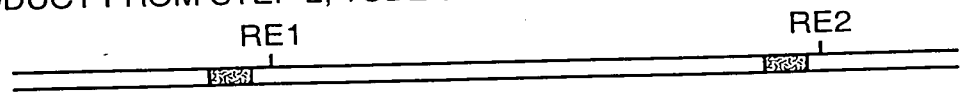
STEP 4: AMPLIFY VIA PCR LIGATED PRODUCTS OF STEP 3 WITH F1 + R4.



STEP 5: DIGEST AMPLIFIED PRODUCT OF STEP 4 WITH RESTRICTION ENDONUCLEASE #2.



STEP 6: LIGATE PRODUCT FROM STEP 5 WITH PRODUCT FROM STEP 2, TUBE 3.



STEP 7: AMPLIFY PRODUCT FROM STEP 6 WITH F1 + R3.

Fig. 4

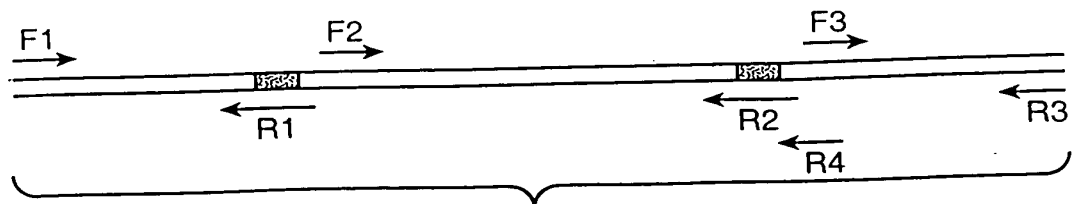


Fig. 5